NOTE ON CARBOLIC ACID AS A FIXATIVE FOR HISTOLOGICAL PREPARATIONS.

By C. Lawrence Herman.

During 1912 whilst working in the Laboratory for Experimental Pathology in Vienna, I was led to investigate the condition of the colloid contents of the thyroid follicle, especially when subjected to different methods of fixation.

Important deductions had been drawn from the appearance of the colloid as seen under the microscope as to its behaviour to stains, and more especially as to being more or less fluid under certain conditions.

This applied particularly to pathological changes, and I wished to determine in how far this was caused by the methods of preparation employed.

I accordingly examined sections of the thyroid after fixation by a large number of different substances in varying dilutions.

On the outbreak of the war I had to leave Vienna very hurriedly and my notes and records had to be left behind and I fear have been lost.

However, without going into details at present, I may say in general I found that most of the fixatives used, including the most frequently employed and customary ones, presented some or other disadvantage, either causing shrinking or vacuolation, or the solution of the colloid wholly or in part; while several interfered with the staining especially of the colloid.

In the case of carbolic acid, however, a good and complete fixation was obtained without shrinking or the production of vacuoles and with very good optical differentiation of cell structures. Moreover, the staining reaction was not interfered with.

On the contrary the various stains used were taken up readily and completely.

Further application of this method to various tissues and organs—the liver, stomach, the abdominal organs generally, and the central nervous system—gave exceedingly gratifying results, especially in cases where rapid penetration and fixation is of advantage, such, for example, as the rat's testicle.

Though carbolic acid was discovered in 1834 (by Runge), it did not come into general use till 1866, when Lord, then Mr., Lister introduced it into practice in the treatment of wounds as a general surgical antiseptic.

Carbolic acid is one of the hydroxyl compounds of benzol, and, like several of this series, is a strong antiseptic and preservative. It is easily soluble in water (1–88 at 16°), and in oils and several other media.

It acts as a general protoplasm poison, and even in dilute solutions kills protozoa quickly unless in extremely attenuated dilutions.

Plant cells are acted on in the same way; ciliated epithelial cells and spermatozoa are killed at once.

It precipitates protein both in solution and in the cells, and does not, like so many substances used in microscopical technique, enter into combination with the albumen to form a fresh compound. It can consequently be washed out with comparative ease.

As a result it penetrates more rapidly and completely into the tissues than metallic or other substances which form insoluble compounds with albumen.

It fixes both the cytoplasm and the nucleus without alteration or distortion, while it leaves all cell structure well defined and clear.

It is of more than ordinary interest to note here that Lister, then engaged in developing his epoch-making life's work, antiseptic surgery, demonstrated in some early experiments the avidity with which epidermis and hair absorbed carbolic acid from its watery solution.

He packed human hair in a test-tube, then covered it with a 5 per cent. carbolic acid solution; in half an hour one-half of the carbolic acid had been extracted from the solution.

In another experiment he packed a glass tube open at both ends with hair, and whilst it stood upright carbolic acid solution was poured in at the upper end. For some time the liquid which flowed from the lower end of the tube contained no carbolic acid at all.

At the same time that carbolic acid is a good fixative it is a preservative, and as such it has been largely used.

I was much interested to learn from Hofrat Professor Paltauf, the distinguished head of the Laboratory for Experimental Pathology, Vienna, that he had examined tissues from an exhumed body which had been soaked in carbolic and buried for some considerable time. The body had been covered with carbolic acid in the hopes of destroying all traces of a crime. He had been much surprised to find the remarkable state of preservation of the tissues, and microscopical sections, especially of the stomach, etc., gave particularly good results.

The cause of this was not fully appreciated at the time, but was made clear on an examination of my preparations.

Largely used in microscopical technique both in histological and bacteriological work, its remarkable power of fixing and preserving tissues for examination under the microscope have not been appreciated, and, if we except the fixative of Pappenheim, in which indeed it is mixed with corrosive sublimate, has not been made use of.

As an antiseptic and preservative Kaiser adds 1·0 carbolic acid to 100·0 gelatine for imbedding, and added to saccharine solution or glycerine jelly it is used for mounting sections.

Weigert uses carbolic-xylol to clear celloidin sections, and it has been mixed with cedar and other oils to act as a clearing agent. Moreover, it is used for dehydrating sections.

Its principal use in microscopy, however, has been as an addition to various aniline dyes to facilitate the staining especially of bacteria, as in the well-known stains, carbol-fuchsin and carbol-methylene-blue, etc.

A solution is easily prepared from Calvert's carbolic acid (I generally use No. 1) or from phenol. The crystals are melted by placing the bottle containing the carbolic in boiling water. Liquefaction quickly takes place. The desired quantity can be readily poured out and measured.

Carbolic acid acts best in 5 per cent. solutions; any lower percentage gives unsatisfactory results.

The preparation to be fixed, preferably small, is left in the fluid from three to six hours; smaller pieces of tissue do not require so long. I have had excellent results with a two hours' fixation, but no harm is done by leaving it longer than six hours in the fluid.

As carbolic acid is freely absorbed by the tissues, the amount of the fluid used must be liberal.

Subsequent washing out is not needed, and the after-treatment follows the usual course—dehydration in the customary manner and clearing by xylol, or preferably by cedar oil, before imbedding.

Briefly, the advantages of carbolic acid as a fixative are:

(1) Rapid penetration and efficient fixation with the least distortion or alteration of tissue.

(2) Good optical differentiation, all cell structures as well as the cell wall remaining well and clearly defined.

(3) The readiness with which good staining is obtained after fixation by this method.

LITERATURE.

EHRLICH.—'Encyklopädie d. Mikroskopischen Technik,' 1910, and article Carbol saure.

CUSHNY.—'Text-book of Pharmacology,' 1910.

GODLEE.—'Life of Lord Lister.'

LEE.—'The Microtomist's Vade-mecum,' seventh edition, 1913.